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PUBLICATION
E.A. Campbell, O. Muzzin, M. Chlenov, J.L. Sun, C.A. Olson, O. Weinman, M. L. Trester-Zedlitz, and S.A. Darst, "Structure of the Bacterial RNA Polymerase Promoter Specificity σ Subunit," *Mol. Cell,* **9**, 527-539 (2002).

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New Insights into Transcription Initiation in Bacteria

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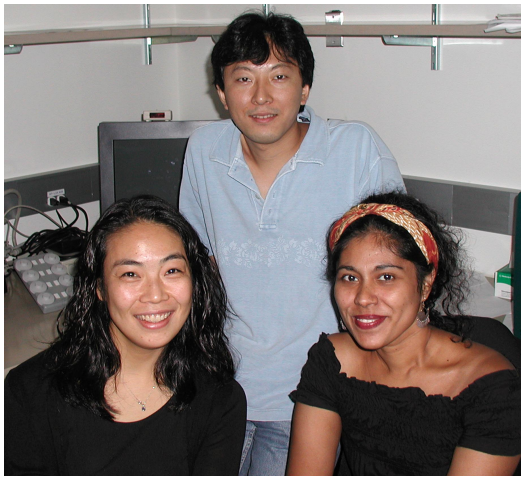
Every cell of a living being contains an instruction manual, the genome, encoded in the form of messages called genes within a double helical molecule made of deoxyribonucleic acid (DNA). This information is read and interpreted in two steps, called transcription, in which the DNA is converted into ribonucleic acid (RNA), and translation, in which the RNA is converted into proteins, used by cells to function properly. Scientists from Rockefeller University have provided structural insights into the key machinery for controlling the initiation of transcription in bacteria.



Some of the authors of the study: (Left to right) Shoko Masuda, Katsuhiko S. Murakami (lead author), and Elizabeth A. Campbell

A genome is the complete "recipe" or set of instructions to make an organism. These instructions are stored in each cell of the organism in the form of a double helical molecule of deoxyribonucleic acid (DNA). The DNA information is read and decoded in two processes, called transcription, in which the DNA is converted into ribonucleic acid (RNA), and translation, in which the RNA is converted into proteins, necessary for the normal functioning of cells.

The conversion of DNA into RNA is performed by a protein called RNA polymerase, which slides along the DNA helix, unzipping the two strands as it goes, synthesizing complementary strands of RNA corresponding to packets of DNA information called genes.

In bacteria, the RNA polymerase has the shape of a 150-angstrom-long crab-claw, with a large cleft between the claws in which the RNA is produced. The RNA polymerase starts synthesizing RNA at defined DNA sequences called promoters, which are recognized by the combination of the RNA polymerase with a protein called the sigma factor. The RNA polymerase with sigma is called the holoenzyme. The sigma factor plays a central role in locating the promoter sequence and in separating the DNA strands, which is required for the RNA polymerase to decode the genetic messages.

Using data from the National Synchrotron Light Source, the Cornell High Energy Synchrotron Source, as well as the Advanced Photon Source, the Rockefeller researchers have provided high-resolution views of the sigma factor structure as well as of the entire holoenzyme bound to a promoter DNA fragment.

Three domains of sigma, labeled σ_2, σ_3, and σ_4, are connected by flexible linkers and are positioned across one face of the RNA polymerase, as shown in **Figure 1**.

One of the linkers connecting the σ_3 and σ_4 domains is particularly long, snaking into the cleft of the RNA polymerase active site and then out again through another channel, the RNA exit channel, that is occupied by the RNA during elongation (**Figure 1**). The linker suggests how sigma may assist in initiating transcription from nucleoside triphosphate (NTP) substrates – the basic units of RNA – by forming part of the initiating substrate-binding site.

The structures mentioned above also provide insights into the curious phenomenon of abortive initiation, in which the initiating RNA polymerase generates large amounts of short RNA fragments while positioned at the promoter, but only rarely moves forward to elongate the entire RNA chain. During initiation, the elongating RNA must displace the sigma linker from the RNA exit channel. Only once in a while, when the RNA manages to push the linker completely out of the RNA exit channel, does abortive initiation cease and the transition into the elongation phase (which follows the initiation phase) can occur.

The two structures above also provide a basis for modeling complexes with the entire promoter (**Figure 2**). Models of the initial 'closed' complex (where the DNA is not melted) and the final 'open' complex (where 14 base-pairs of DNA have been melted) provide a framework for designing further experiments to understand how the RNA polymerase operates.

Additional Publications:
K.S. Murakami, S. Masuda, and S.A. Darst. "Structural Basis of Transcription Initiation: RNA Polymerase Holoenzyme at 4 Å Resolution," *Science,* **296**, 1280-1284 (2002).

K.S. Murakami, S. Masuda, E.A. Campbell, O. Muzzin, and S.A. Darst. "Structural Basis of Transcription Initiation: An RNA Polymerase Holoenzyme/DNA Complex, *Science,* **296**, 1285-1290 (2002).

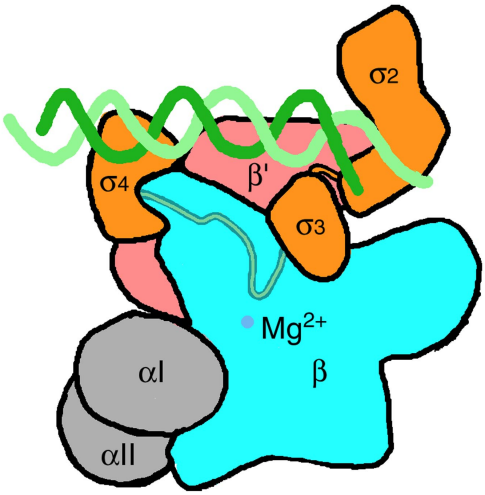


Figure 1. *Cartoon model of a complex of the RNA polymerase holoenzyme and a fragment of the DNA promoter. The sigma subunit domains are labeled σ_2, σ_3, and σ_4, with the linker connecting σ_3 and σ_4 extending through the main RNA polymerase channel near the active site containing magnesium (purple sphere).*

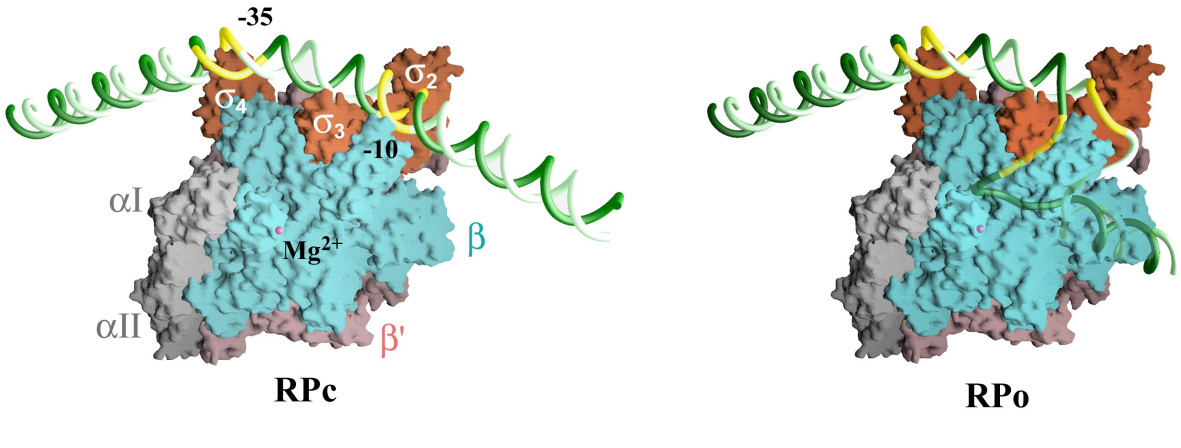


Figure 2. *Models of RNA polymerase closed (RP_c) and open (RP_o) complexes with promoter DNA.*